EFFECTS OF TRYPANOCIDAL DRUGS ON THE REPLICATION AND FUNCTION OF KINETOPLAST (MITOCHONDRIAL) DNA IN TRYPANOSOMES

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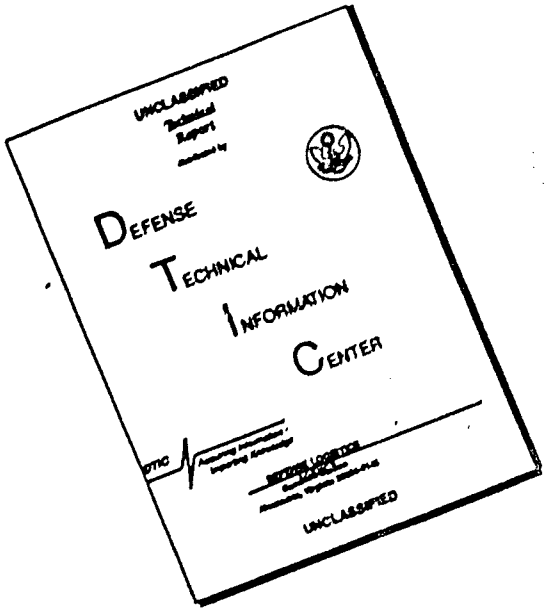

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3. Suramin is a potent inhibitor of E. coli RNA polymerase. The inhibition can be reversed by increasing concentrations of DNA and enzyme;
4. Isolated mitochondria have RNA polymerase activity;
5. Action spectral evidence has conclusively demonstrated the presence of cytochrome o and cytochrome aa_3 in T. mega, B. culicis and L. tarentolae.

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Annual Progress Report

George C. Hill

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ABSTRACT

The purpose of our studies has been to determine the effects of trypanocidal drugs on the replication of kinetoplast (mitochondrial) DNA. We have also been interested in determining the mode of action of trypanocidal drugs. Our approach to resolving this problem includes investigating various enzymes in host and trypanosomes, studying the effects of trypanocidal drugs on enzyme systems isolated from trypanosomes and studying the structure and transcription ability of purified kinetoplast DNA. We are interested in determining the reason for the unique selective toxicity of known trypanocidal drugs.

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FORWARD

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care", as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences - National Research Council.

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APPROACH TO THE PROBLEM

The numerous and necessarily speculate points raised in our discussion of trypanocidal drug action are now mostly capable of experimental verification with currently available techniques and data from related fields of cell biochemistry. Given the necessary attention, this largely neglected but important field should yield results of considerable value, not only for an understanding both of trypanocidal drug action and of trypanosomal metabolism, but for cell biology in general. This is the purpose of this contract. So far as trypanocidal drug design is concerned, the era of intelligent empericism is unlikely to be superseded until the balance of effort and expenditure on drug production is adjusted more favorably in the direction of research on the metabolism of trypanosomes and on the mechanisms whereby existing drugs exert their specific effects.

Our approach to the problem of developing new trypanocidal agents includes investigating:

1. The effects of trypanocidal drugs on enzyme systems isolated from trypanosomes;
2. Detailed comparisons of homologous enzymes in host and trypanosomes;
3. Unique cell components or metabolic pathways in parasites;
4. The basis of the selective toxicity of known drugs.

THE BACKGROUND

African trypanosomiasis is confined to Africa by the distribution of its vectors. T. gambiense infection occurs over a broad belt from Senegal in the west to the great lakes Victoria, Albert, Benguelo, and Tanzania in the east, extending as far sough as north Angola. T. rhodesiense is located in east and central Africa and is scattered over a longitudinal band from the south Sudan to Mozambique. The importance of trypanosomiasis of man and animals in Africa has been summarized recently by Kershaw (1) in the statement:

> "The World Health Organization in determining the ten major health problems facing mankind places trypanosomiasis of man and his domestic animals high on the list along with malaria, cancer and heart diseases."

The need for new trypanocides cannot be overemphasized. At present, chemotherapy of African trypanosomiasis is dependent on a relatively small number of synthetic drugs (Figure 1). Suramin (I) and pentamidine (II) are used for prophylaxia and treatment of early stages of the disease in man. Organic arsenicals such as tryparsamide (III) and melaminyl compounds (IV) are used for advanced cases, when trypanosomes have invaded the central nervous system. The disease in cattle and other domestic animals is controlled by quaternary ammonium trypanocides (Antrycide (V), Ethidium (VI), Prothidium (VII), and related drugs) and by the aromatic diamidine, Berenil (VIII). As pointed out recently by Newton (2), resistance has been reported to occur against all these drugs and development of resistance to one compound is often accompanied by cross-resistance to another.

In human trypanosomiasis, there is still an urgent requirement for a cheap, simply administered and well-tolerated, preferably "one-shot" drug which would be as effective a prophylactic as pentamidine and active therapeutically against all stages of the infection in both Gambian and Rhodesian sleeping sickness. It should also be incapable of inducing drug resistance and active also against strains with acquired resistance to other drugs.

Possibly the two requirements of prolonged tissue retention (for prophylaxis) and ability, to penetrate into the central nervous system are mutually exclusive, but with increasing knowledge of the structure and function of the so-called "blood-brain barrier", this problem should not be insuperable.

In none of the active drugs is the mode of action precisely known. An excellent review on the mode of action of trypanocidal drugs has been prepared by Williamson (3). More recent studies have suggested that berenil and ethidium bromide form complexes with DNA. In the case of ethidium, it is clear this drug is a potent and selective inhibitor of DNA synthesis. It has been shown by several investigators that both phenanthridines and acridines combine with DNA by the heterocyclic chromophore of the drug molecules becoming inserted, or intercalated, between the adjacent base pairs in the double-stranded helix of DNA. Such intercalation is achieved by a partial uncoiling of the DNA helix which results in the base pairs above and below the bound drug molecule becoming separated by twice their normal distance (2).

More recently, it has been shown that phenanthridines also bind to supercoiled DNA of the type found in certain tumor viruses, mitochondria of

many cell types and kinetoplast of trypanosomes. There is evidence that these drugs bind preferentially to such DNA in vivo and give rise to dyskinetoplastic trypanosomes (4) and "petite mutants" of yeast (5). The molecular basis of this preferential binding is not yet fully understood. The findings that have been observed could adequately explain the growth inhibitory activity of phenanthridine drugs but it remains to be established whether their primary action on bloodstream forms of trypanosomes is to inhibit DNA synthesis.

Berenil, an aromatic diamidine, has been shown to interact with DNA and can selectively block kinetoplast replication (6, 7). The earliest reported effect observed of berenil is the localization in the kinetoplast of T. brucei. This has been detected by ultraviolet microscopy within an hour of a curative dose being injected intraperitoneally into infected mice and within seconds of the drug being added to an in vitro suspension of trypanosomes (7). Recent work has shown that berenil can form complexes with purified DNA, but in contrast to phenanthridines, there is good evidence that the complexes are not formed by intercalation (8).

A detailed examination of kinetoplast DNA isolated from berenil-treated T. cruzi has shown that many of the small circular DNA molecules appear as branched structures (6). These forms, which are thought to be replicative molecules, are rarely seen in control preparations, suggesting that berenil does not block the replication of kinetoplast DNA at initiation but binds preferentially to certain specific points in the circular DNA molecule. As for phenanthridines, it cannot be said what is the primary effect of berenil or other diamidines on trypanosomes.

The mode of action of suramin remains enigmatic even after more than a half century of use. In vitro exposure of trypanosomes to suramin at concentrations as low as 10^{-5} M is known to reduce their infectivity whereas concentrations as high as 10^{-2} M do not affect the motility or respiration of cells. As would be expected from its structure, the drug binds avidly to basic proteins and is known to inhibit many isolated enzymes (3). The most sensitive enzymes examined appear to be hyaluronidase, inhibited at 10^{-5}-10^{-6} M, fumarase, inhibited at ca. 10^{-7} M, urease at pH 5 (ca. 10^{-4} M), hexokinase (10^{-4}-10^{-5} M), and RNA polymerase (10^{-5} M) (9). The ready absorption of this drug by plasma proteins may well account for the long retention time of the compound in man and animals and contribute to its value as a prophylactic agent. The question of how a molecule as large as suramin enters trypanosomes is an interesting one and it seems possible that, when protein bound, suramin actively stimulates pinocytosis. As with the other drugs that we have discussed, there is evidence that suramin becomes localized in lysosomes. Again whether this is important to the trypanocidal action of the drug or whether it is a secondary phenomenon is unknown.

RESULTS AND DISCUSSION

The results shall be discussed in sections containing specific areas that we have investigated. All the topics investigated relate to the specific objectives of the project.

A. Homology and Kinetoplast DNA and Nuclear DNA

One of the specific objectives is related to determining the functional capacity of K-DNA. In this regard we have attempted to study the homology of the RNA synthesized *in vitro* which is complementary to the *C. fasciculata* K-DNA used as template. Complementary RNA was synthesized with *C. fasciculata* RNA polymerases I and II and *E. coli* RNA polymerase. The standard reaction mixture was increased 20 fold. Purification of the complementary RNA was following procedures outlined by Tabak and Borst (10). The results of these hybridization studies are presented in reference 9. It was determined that the complementary RNA did not hybridize with *C. fasciculata* nuclear DNA (N-DNA) but did hybridize with K-DNA.

Regardless of the RNA polymerase employed, there was no difference in the ability of the complementary RNA synthesized from *C. fasciculata* K-DNA to hybridize with *C. fasciculata* K-DNA or N-DNA. The complementary RNA hybridized 42-45% with K-DNA but only 1% with N-DNA.

Our results would suggest little homology between K-DNA and N-DNA. The hybridization procedures employed detected little homology between *C. fasciculata* K-DNA and N-DNA, regardless of the RNA polymerase enzyme used to synthesize the complementary RNA (9).

B. Preparation of Purified Kinetoplast and Nuclear DNA

Our initial efforts were devoted towards obtaining a pure preparation of C. fasciculata and Leptomonas sp. K-DNA and N-DNA. This was obtained by procedures previously described (9). The purity of the K-DNA and N-DNA preparations can be observed in Figure 2 of Ref. 9. It can clearly be seen that both preparations have only one component of DNA present and is not contaminated by other cellular DNA. We are proceeding to use Leptomonas sp. DNA because the organisms are easier to break in order to isolate the DNA. They break at 100 psi. In contrast, C. fasciculata is very resistant to breakage. In addition, Leptomonas sp. is much more sensitive than C. fasciculata to the trypanocidal drugs whose mode of action we wish to study. We first observed this high sensitivity of trypanocidal drugs to this organism (11).

C. Preparation of E. coli DNA and RNA Polymerases

These enzymes have been prepared by several procedures. The E. coli DNA polymerase that we are using has been prepared by the method of Jovin et al. (12). The E. coli RNA polymerase has been prepared by the procedure of Chamberlin and Berg (13). The previous research experience of Dr. Dalbow has been extremely helpful in preparing large and purified quantities of these enzymes.

D. Preparation of Mitochondrial RNA polymerases from C. fasciculata

This task has been extremely difficult but some preliminary success has been obtained. Several approaches have been tried. Initially, we studied the ability of mitochondria isolated from C. fasciculata to

synthesize RNA. Results from three experiments are summarized in Table 1. The results demonstrate the ability of these mitochondria to synthesize RNA.

Mitochondrial RNA polymerase is membrane-bould and its isolation and purification usually leads to its instability. We have thus attempted to isolated a K-DNA and RNA polymerase complex from C. fasciculata. We hope that by isolating a transcription complex that this would be more stable after isolation. The isolated transcription complex was prepared by modifying the procedure used for isolating the K-DNA. No proteolytic enzymes or RNase were added during the isolation procedures. Advantage was taken of the property of the kinetoplast network to be of such large molecular weight (e.g. 10 X 10^{10} daltons), so as to permit the separation of the K-DNA and RNA polymerase from the N-DNA by sucrose density gradient centrifugation. The results of a preliminary experiment are seen in Table 2. These results demonstrate the sensitivity of the complex to DNase and RNase. In addition the synthesis of RNA was sensitive to rifampicin, an inhibitor of mitochondrial RNA polymerase.

While this procedure has produced positive results, we have considered isolating an intact mitochondrial preparation from Leptomonas sp. Then we shall evaluate the RNA synthesis activity in the presence of trypanocidal drugs. In relationship to our specific objectives, we shall then be prepared to characterize the properties of the RNA synthesized in vitro by the K-DNA.

E. Effects of Various Trypanocidal Drugs on K-DNA-Directed RNA Synthesis

The purified E. coli DNA polymerases have been used to study in vitro the K-DNA-directed RNA synthesis. As can be seen by this report, both Leptomonas sp. and C. fasciculata N-DNA and K-DNA have been used. The properties of the enzyme system have been carefully studied. The results of three experiments are summarized in Figures 2 and 3. The synthesis of RNA and K-DNA is dependent on the amount of K-DNA present and the amount of enzyme. The concentration of K-DNA used in these studies is 0.4 µg/60 µl of reaction or 13.3 µg/ml. The concentration of E. coli RNA polymerase used was 0.05 µg/60 µl. Table 2 in reference 9 presents the results supporting evidence for the K-DNA-directed RNA synthesis. The reaction is dependent on DNA, nucleotides and Mn^{++}.

The effects of three trypanocidal drugs have been studied in order to observe their effects on K-DNA directed RNA synthesis. These drugs are ethidium bromide, berenil and suramin. The effects of these drugs can be seen in Figure 4. The drug effect at the lowest concentrations is ethidium bromide. The inhibition produced by berenil and suramin can also be observed.

The inhibition of ethidium bromide and berenil was investigated to see if a preferential inhibition was observed for circular (K-DNA) or linear (N-DNA) (Table 3). No preference for circular DNA was observed. The effects of these trypanocidal drugs on the K-DNA-directed RNA synthesis was studied when the K-DNA was native as well as alkaline denatured. The average of results for three experiments for each drug can be observed in Figures 4-6. There is a slight preference for

native K-DNA by ethidium bromide (Figure 5). Suramin inhibition is significantly greater with native K-DNA (Figure 7). In the case of berenil, a greater inhibition of K-DNA-directed RNA synthesis occurs when the template is denatured.

The mode of action of these drugs is not known. As previously mentioned in the background section, while these drugs are effective and inhibit trypanosomes, there remains to be a clear identification of their mode of action. A continued investigation of their biochemical effects on trypanosomes is necessary if we are to identify their chemotherapeutic action.

F. Effects of Suramin on K-DNA-Directed RNA Synthesis

As noted in Figures 4 and 6, the trypanocidal compound suramin inhibits K-DNA-directed RNA synthesis. This inhibition is markedly decreased if the K-DNA is denatured and then used as template (Figure 7). We have devoted some time to studying the mode of action of this drug and how its inhibition can be reversed.

The kinetics of RNA synthesis in the absence and presence of suramin are presented in Figure 8. RNA synthesis by DNA-dependent RNA polymerase can be separated into three distinct processes:

1. Binding of enzyme to specific sites on the DNA template to form a DNA-enzyme complex;
2. Initiation of RNA synthesis by reaction of the DNA-enzyme complex with purine nucleotides to form a stabilized initiation complex;
3. Polymerization of four kinds of nucleotides into RNA chain under the direction of the DNA template.

As can be seen in Figure 8, suramin inhibits the reaction if added after initiation has occurred (at plus 5 minutes). Thus, suramin must be inhibiting beyond the initiation step of RNA synthesis and probably at the polymerization level of transcription of K-DNA. It would thus be preventing the polymerization of the nucleotides into an RNA chain under the direction of the K-DNA template. Whether it also inhibits at the binding or initiation levels cannot be conclusively demonstrated in this experiment.

The next question we have tried to answer is what type of inhibition is the suramin inhibition. The decrease in inhibition when the K-DNA is denatured suggests that the secondary structure of the K-DNA is important in suramin inhibition. Using native DNA we have determined that K-DNA reverses the inhibition of suramin (Table 4 and Figure 9) and that this inhibition is noncompetitive for the K-DNA (Figure 10). The present inhibition of the suramin on the RNA polymerase is seen in Figure 11.

Suramin inhibition of RNA synthesis could also be reversed by increasing concentrations of the enzyme. The results of these experiments can be seen in Table 5 and Figures 12-15. Evidence is presented in Figure 13 that the inhibition of suramin for the RNA polymerase is competitive for enzyme. The inhibition is similar to the type of inhibition reported for kanchanomycin (14). Kanchanomycin, an antibiotic produced by a Streptomyces species, is also a non-competitive inhibitor for DNA and a competitive inhibitor for RNA polymerase. Suramin inhibits the K-DNA-directed RNA synthesis but is most effective

if it has a chance to react with the RNA polymerase before the K-DNA. In Table 6 are presented the results of three experiments. If the enzyme and K-DNA are pre-incubated, the inhibition by suramin is 30% less than if suramin is pre-incubated with the K-DNA or enzyme. Our hypothesis at the present time is that suramin competes with the K-DNA for initiation sites on the RNA polymerase. We also believe at high concentrations of suramin (0.25 mM) some allosteric inhibition of the enzyme occurs. Whether the mode of action of suramin *in vivo* is on the DNA and RNA synthesis in trypanosomes has not yet been determined.

G. Effects of Trypanocidal Drugs on K-DNA-Directed DNA Synthesis

The effects of ethidium bromide, berenil and suramin on K-DNA-directed DNA synthesis is presented in Figure 15. All three drugs observed inhibited K-DNA replication *in vitro*. Again no preference for circular DNA was observed. All three drugs observed inhibited the K-DNA-directed DNA synthesis at lower levels than they inhibited K-DNA-directed RNA synthesis. The concentration of DNA polymerase (0.008 µg/60 µl) used was approximately 6-fold greater than the RNA polymerase. Thus the greater inhibition of these drugs on the DNA polymerase reflects a greater sensitivity of DNA synthesis to ethidium bromide, berenil and suramin.

H. Identification of Cytochrome o by Action Spectra

In order to observe the effects of trypanocidal drugs on the electron transport system, it is necessary to know all the components of the cytochrome system in cyanide-sensitive trypanosomes. We have

conclusively identified, by photochemical relief of CO-inhibition of respiration, that cytochrome o is present in trypanosomes (15). The organisms included Trypanosoma mega, Blastocrithidia culicis and Leishmania tarentolae. The electron system is thus branched with two terminal oxidase (16). We are now in a position to determine what the effects are of trypanocidal drugs on the formation of the electron transport system.

CONCLUSIONS

The first year of this contract has been productive. The main conclusions are:

1. Little homology exists between C. fasciculata purified kinetoplast and nuclear DNA;
2. The trypanocidal drugs berenil, ethidium bromide, antrycide and suramin inhibit E. coli DNA and RNA polymerases;
3. Suramin is a potent inhibitor of E. coli RNA polymerase. The inhibition can be reversed by increasing concentrations of DNA and enzyme;
4. Isolated mitochondria have RNA polymerase activity;
5. Action spectral evidence has conclusively demonstrated the presence of cytochrome o and cytochrome aa_3 in T. mega, B. culicis and L. tarentolae.

RECOMMENDATIONS

As previously noted, the mode of action of trypanocidal drugs is not known. We recommend a continued effort to determine the mode of action of some of the more effective drugs including berenil, antrycide and suramin. In these studies, we believe it is important and essential to couple the investigation of the mode of action of these drugs in vitro with their effects on pathogenic trypanosomes in mice or rats. These studies are essential if we are to develop a more rational drug development approach.

We would recommend that careful consideration be given to the systems to be used. It should be remembered that there is still no in vitro model for the in vivo infection in mice or rats of trypanosomiasis. In order to determine the mode of action of the drugs, one must consider the drug effects on the host-parasite system.

Support should be considered for studying the techniques required for maintaining the bloodstream forms in vitro. At the present time, this is not possible for any significant period of time. In addition, more research is needed on the pathobiology of trypanosomiasis. Little information is available on the pathogenesis and pathology of this group of diseases which make up the tsetse-transmitted animal trypanosomiasis of Africa.

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TABLE 1.

RNA Synthesis Capacity of Mitochondrial Preparation from C. fasciculata

Additions	Percent Inhibition
Preparation + RNase (100 μg/ml)	47.8
+ DNase (100 μg/ml)	11.1
+ rifamycin (0.25 mM)	29.9
+ Suramin (0.25 mM)	48.5
+ acriflavine (0.25 mM)	-
+ ethidium bromide (0.25 mM)	-

20 μl of the mitochondrial preparation from C. fasciculata was added to a total volume of 60 μl of reaction mixture. The inhibitors was pre-incubated at 4°C for 5 minutes with the reaction mixture and then the reaction was allowed to proceed for 30 minutes at 37°C. Acriflavine stimulated the reaction 13% and ethidium bromide caused a 16% stimulation.

TABLE 2.

RNA Synthesis Capacity of C. fasciculata RNA Polymerase - K-DNA Complex

Additions	Percent Inhibition
Complex + DNase (100 µg/ml)	100
+ RNase (100 µg/ml)	100
+ Rifampicin (0.25 mM)	41.5
+ Suramin (0.25 mM)	0
+ α-amanitin (40 µg/ml)	28.6
+ actinomycin D (0.25 mM)	40.6

20 µl of the transcription complex was added to a total volume of 60 µl reaction mixture. The control counts were 327 cpm. The reaction with inhibition was pre-incubated at 4°C for 5 minutes and then incubated at 37°C for 30 minutes.

TABLE 3.

Effect of Berenil and Ethidium Bromide on the E. coli RNA Polymerase Reaction Using Leptomonas sp. K-DNA and N-DNA.

Drug Concentration (mM)	Percent Inhibition	
	K-DNA	N-DNA
Berenil		
0.25	80.5	72.0
0.06	36.6	42.8
0.03	18.2	41.5
Ethidium Bromide		
0.06	90.0	87.5
0.03	85.3	73.4
0.003	3.7	17.5

0.4 µg of Leptomonas sp. native K-DNA was present. The final concentration of the E. coli RNA polymerase was 0.05 µg/50µl. The total reaction mixture including drug and enzyme was incubated at 4°C for 5 minutes and then at 37° for 30 minutes.

TABLE 4.

Effect of Increasing Concentrations of C. fasciculata K-DNA on the Inhibition of E. coli RNA Polymerase Reaction by Suramin

DNA Concentration (µg/60 µl)	Suramin Concentration (mM)	Percent Inhibition
0.4	-	-
0.4	0.08	88.6
1.2	0.08	72.6
3.6	0.08	61.9

The DNA concentration refers to the amount of native C. fasciculata K-DNA in the 60 µl reaction mixture. The final concentration of enzyme was 0.05 µg/60µl. Suramin was incubated at 4°C for 5 minutes with the total reaction mixture and then the reaction was allowed to continue for 30 minutes at 37°C.

TABLE 5.

Effect of Increasing the Concentration of Enzyme on Suramin Inhibition of *E. coli* RNA Polymerase Reaction

Enzyme Concentration (μg/60 μl)	Suramin Concentration (mM)	Percent Inhibition
0.05	-	-
0.05	0.125	97.7
0.30	0.125	71.0

0.4 μg of native *Leptomonas* sp. K-DNA was used as template. Suramin was incubated at 4°C for 5 minutes with the total reaction mixture and then the reaction was allowed to continue for 30 minutes at 37°C.

TABLE 6.

Effect of the Order of the Addition of Suramin, K-DNA and Enzyme on the Inhibition of the E. coli RNA Polymerase Reaction

Reaction Mixture	Component(s) Added at Zero Time	Percent Inhibition
Control	DNA + Enzyme	0
Control	DNA + Enzyme + Suramin	60.3
Suramin + K-DNA	Enzyme	93.2
Suramin + Enzyme	K-DNA	90.6
Enzyme + K-DNA	Suramin	63.6

Various components were added and incubated with the reaction mixture for 5 minutes at 4°C. The final component was then added and the reaction began by incubation at 37°C for 30 minutes. 0.4 µg of Leptomonas sp. K-DNA was present. The final concentration of E. coli RNA polymerase was 0.05 µg/60 µl.

FIGURE LEGENDS

Figure 1. Structure of various trypanocidal drugs.

Figure 2. Effects of increasing concentrations of Leptomonas sp. K-DNA on RNA synthesis. The concentration of E. coli RNA polymerase was 0.05 µg/60 µl.

Figure 3. Effects of increasing enzyme concentrations on the Leptomonas sp. K-DNA-directed synthesis of RNA. The K-DNA concentration was 0.4 µg/60 µl.

Figure 4. Effects of various concentrations of ethidium bromide, suramin and berenil on Leptomonas sp. K-DNA-directed RNA synthesis. The concentration of E. coli RNA polymerase was 0.05 µg/60 µl. The concentration of K-DNA was 0.4 µg/60 µl.

Figure 5. Effects of ethidium bromide on Leptomonas sp. K-DNA-directed RNA synthesis. Both native and alkaline-denatured K-DNA was added. The concentration of E. coli RNA polymerase was 0.05 µg/60 µl. The DNA concentration was 0.4 µg/60 µl.

Figure 6. Effects of berenil on Leptomonas sp. K-DNA-directed RNA synthesis. Both native and alkaline-denatured K-DNA was added. The concentration of K-DNA used was 0.4 µg/60 µl. The concentration of E. coli RNA polymerase was 0.05 µg/60 µl.

Figure 7. Effects of suramin on Leptomonas sp. K-DNA-directed RNA synthesis. Both native and alkaline-denatured K-DNA was added. The concentration of K-DNA used was 0.4 µg/60 µl. The concentration of E. coli RNA polymerase was 0.05 µg/60 µl.

Figure 8. Kinetics of the suramin inhibition of Leptomonas sp. K-DNA-directed RNA synthesis. 0.08 mM suramin was added just prior to incubation and at 5 minutes after incubation.

Figure 9. Effects of increasing concentrations of Leptomonas sp. K-DNA on suramin inhibition of RNA synthesis. The concentration of E. coli RNA polymerase was 0.05 µg/60 µl.

Figure 10. Lineweaver - Burk plot of suramin inhibition of Leptomonas sp. K-DNA-directed RNA synthesis as K-DNA concentration is increased.

Figure 11. Effect of increasing K-DNA concentrations on the inhibition of Leptomonas sp. K-DNA-directed RNA synthesis by suramin. The E. coli RNA polymerase concentration was 0.05 µg/60 µl.

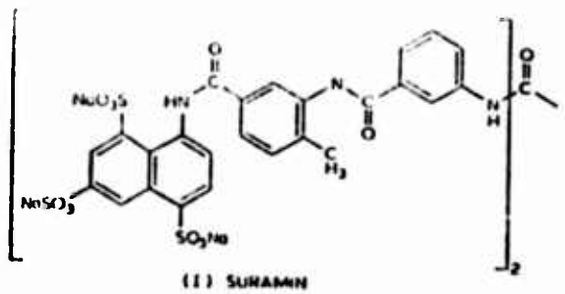
Figure 12. Effect of increasing enzyme concentration on the inhibition by suramin of Leptomonas sp. K-DNA-directed synthesis of RNA. The concentration of K-DNA was 0.4 µg/60 µl.

Figure 13. Lineweaver - Burk plot of suramin inhibition of Leptomonas sp. K-DNA-directed RNA synthesis as enzyme concentration is increased.

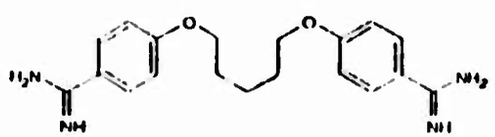
Figure 14. Effect of increasing concentrations of enzyme on suramin inhibition of Leptomonas sp. K-DNA-directed RNA synthesis. The K-DNA concentration was 0.4 µg/60 µl.

Figure 15. Effect of various concentrations of ethidium bromide, berenil and suramin on the Leptomonas sp. K-DNA-directed DNA synthesis. The concentration of K-DNA was 0.4 µg/60 µl. The concentration of E. coli DNA polymerase was 0.008 µg/60 µl.

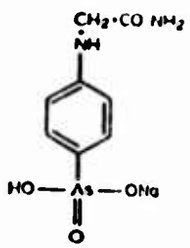
FIGURE 1



(I) SURAMIN

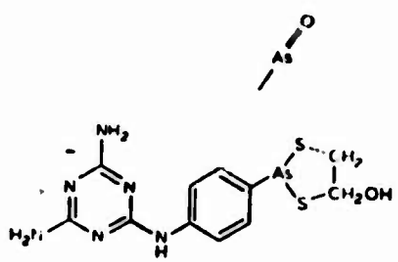


(II) PENTAMIDINE

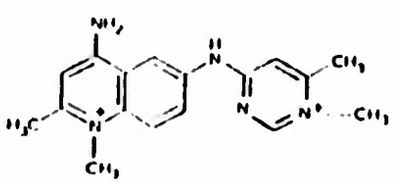


(III) TRYPARSAMIDE

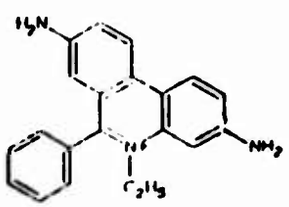
MELARSEN OXIDE



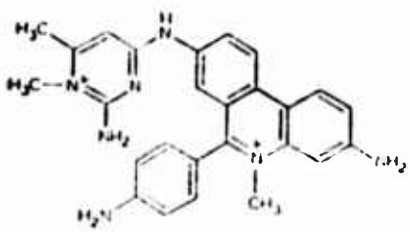
(IV) MEL B



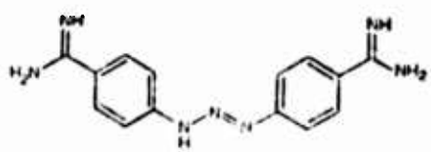
(V) ANTRYCIDE



(VI) ETHIDIUM



(VII) PROTHIDIUM



(VIII) BERENIL

Figure 2

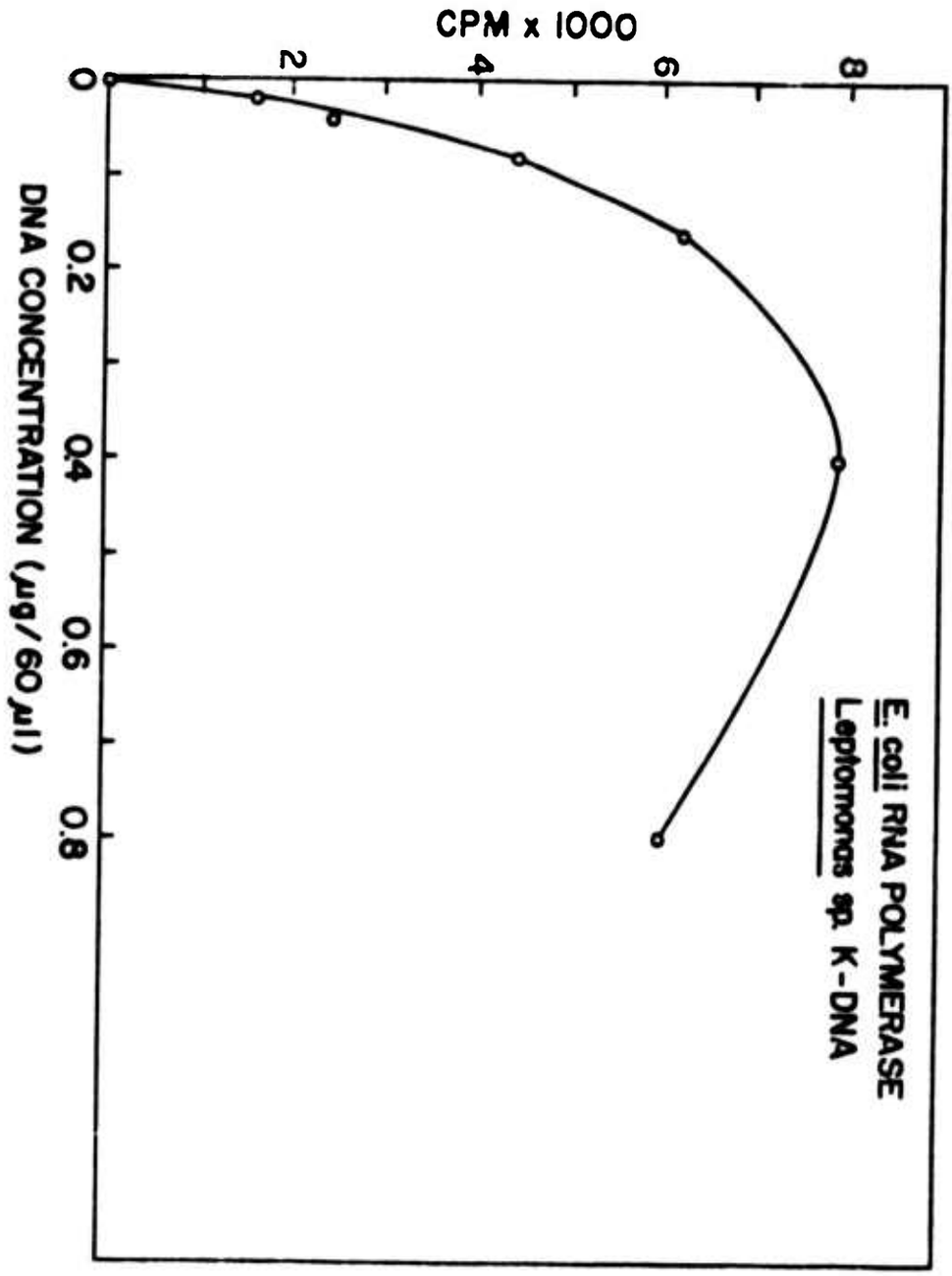

CPM x 1000
0
2
4
6
8
0.2
0.4
0.6
0.8
DNA CONCENTRATION (µg/60 µl)
E. coli RNA POLYMERASE
Leptomonas sp. K-DNA

Figure 3

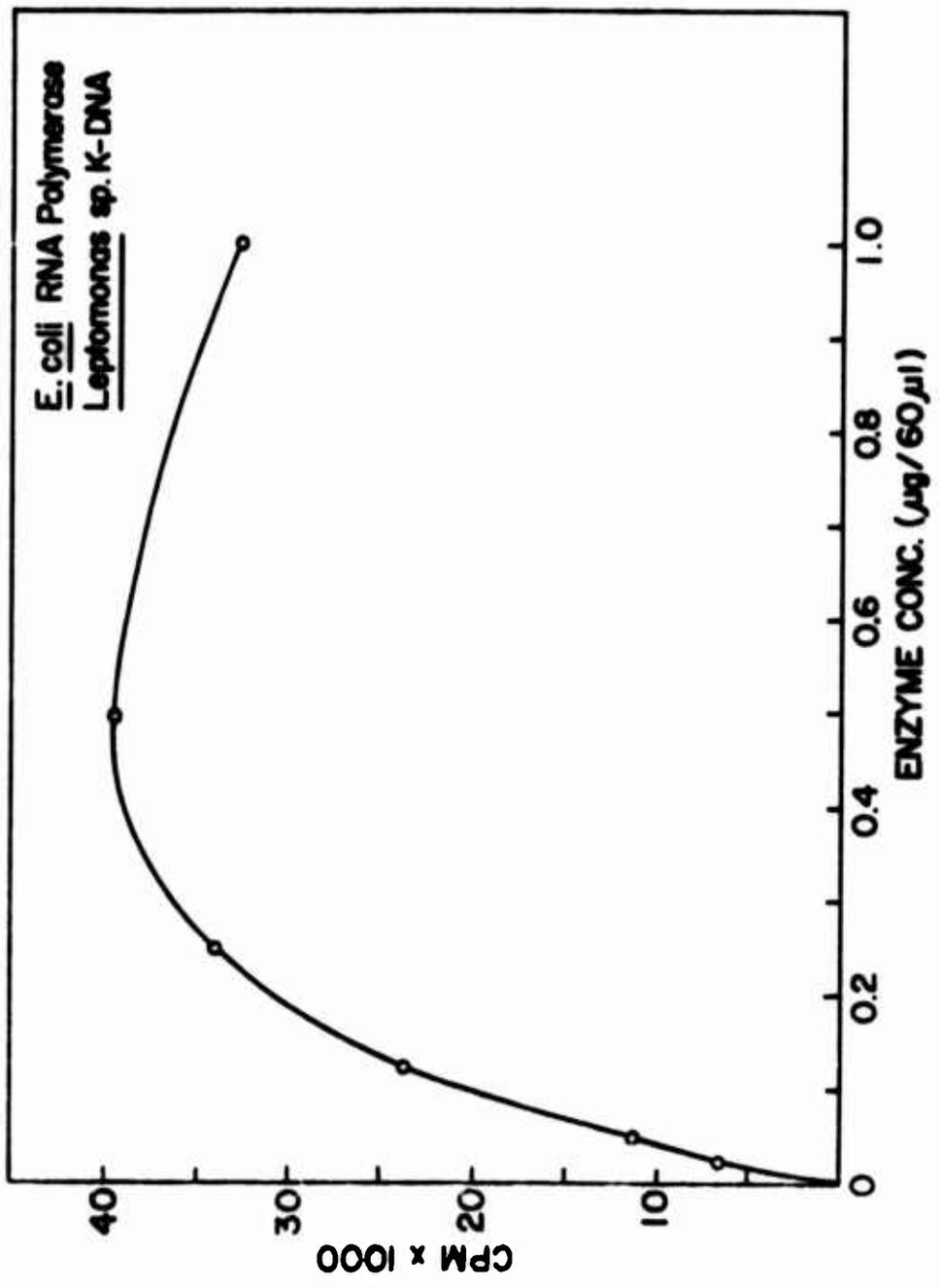

E. coli RNA Polymerase
Leptomonas sp. K-DNA
CPM x 1000
40
30
20
10
0
0.2
0.4
0.6
0.8
1.0
ENZYME CONC. (µg/60µl)

Figure 4

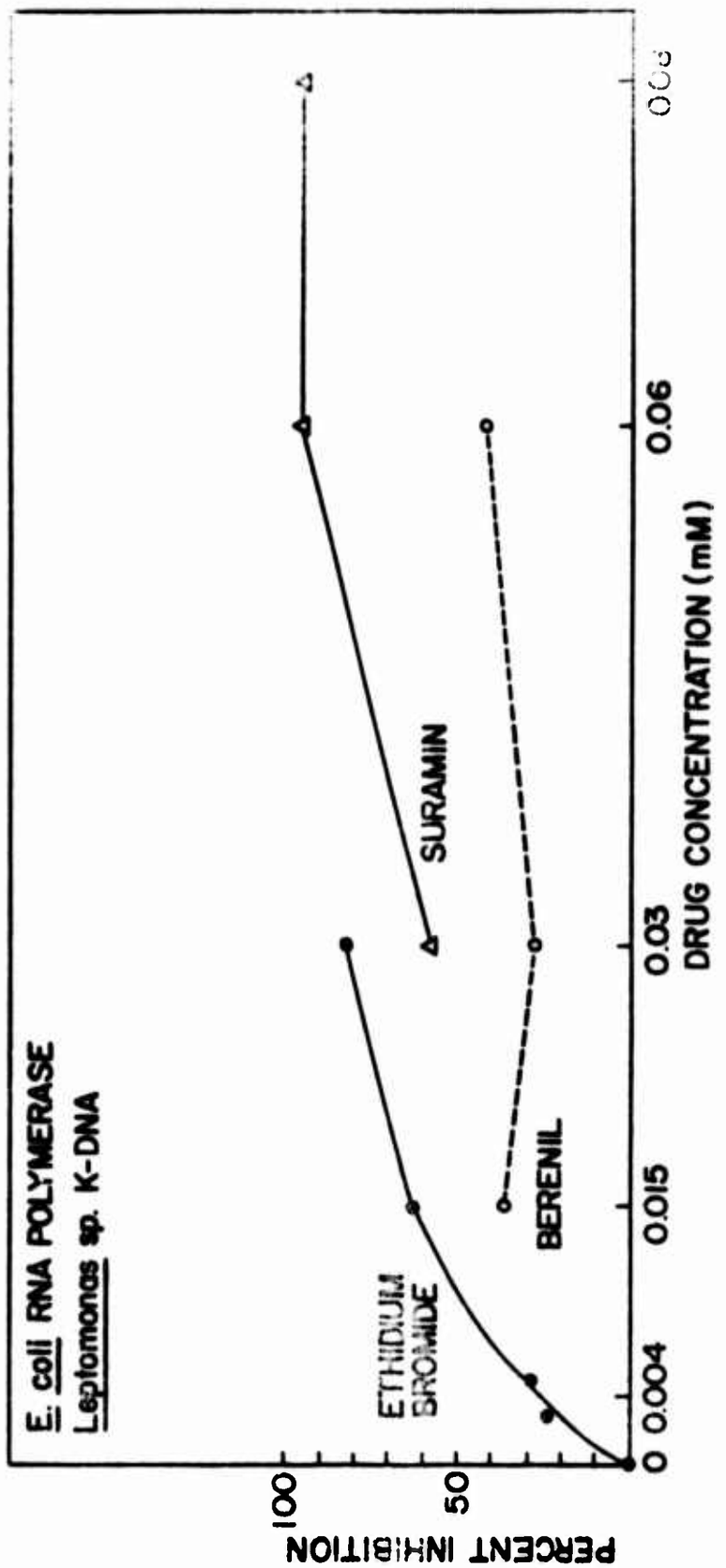

E. coli RNA POLYMERASE
Leptomonas sp. K-DNA
ETHIDIUM BROMIDE
SURAMIN
BERENIL
PERCENT INHIBITION
100
50
0
0.004
0.015
0.03
0.06
DRUG CONCENTRATION (mM)

FIGURE 5

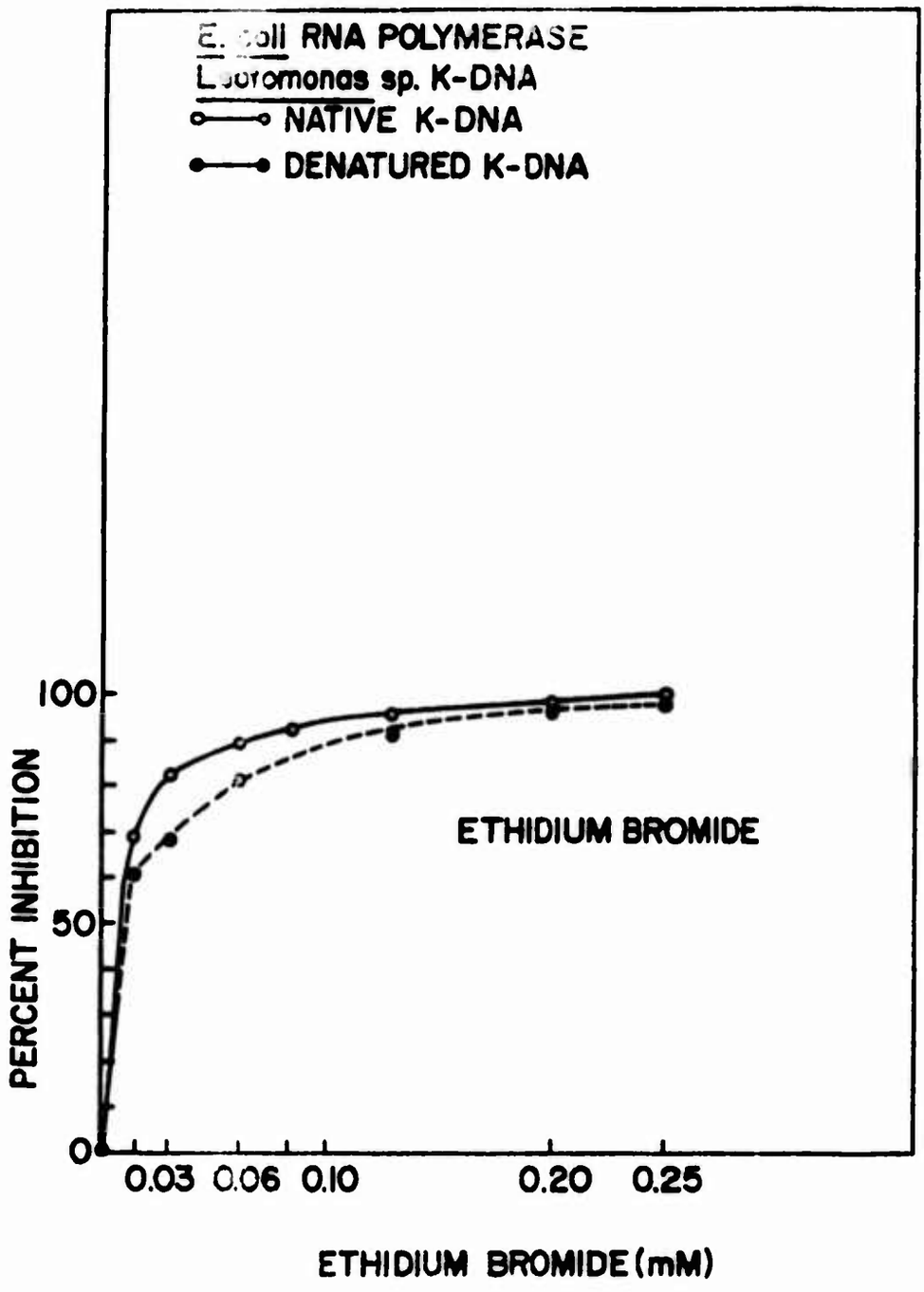

E. coli RNA POLYMERASE
Leuromonas sp. K-DNA
NATIVE K-DNA
DENATURED K-DNA
ETHIDIUM BROMIDE
PERCENT INHIBITION
100
50
0
0.03
0.06
0.10
0.20
0.25
ETHIDIUM BROMIDE (mM)

FIGURE 6

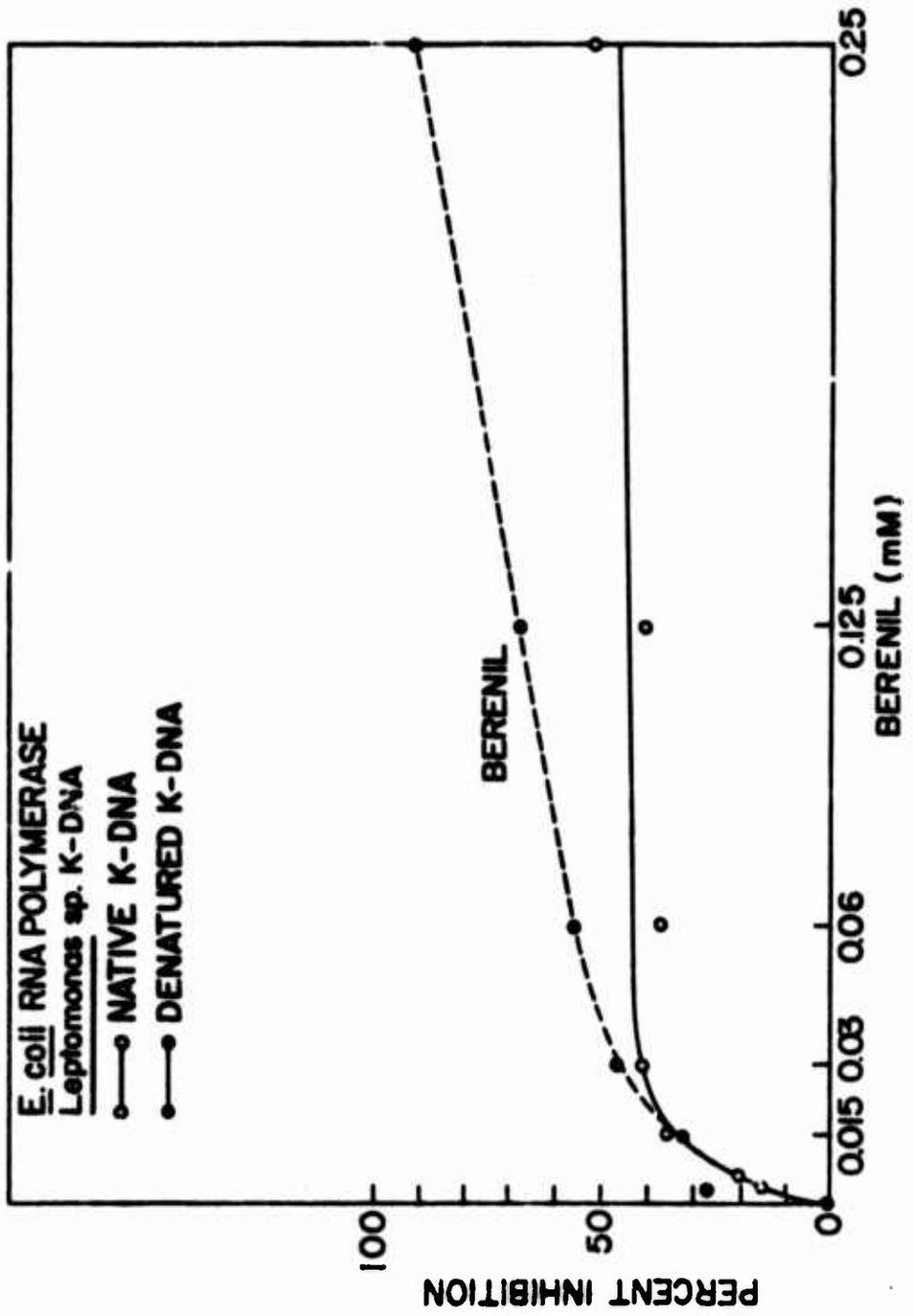

E. coli RNA POLYMERASE
Leptomonas sp. K-DNA
NATIVE K-DNA
DENATURED K-DNA
BERENIL
PERCENT INHIBITION
100
50
0
0.015
0.03
0.06
0.125
0.25
BERENIL (mM)

FIGURE 7

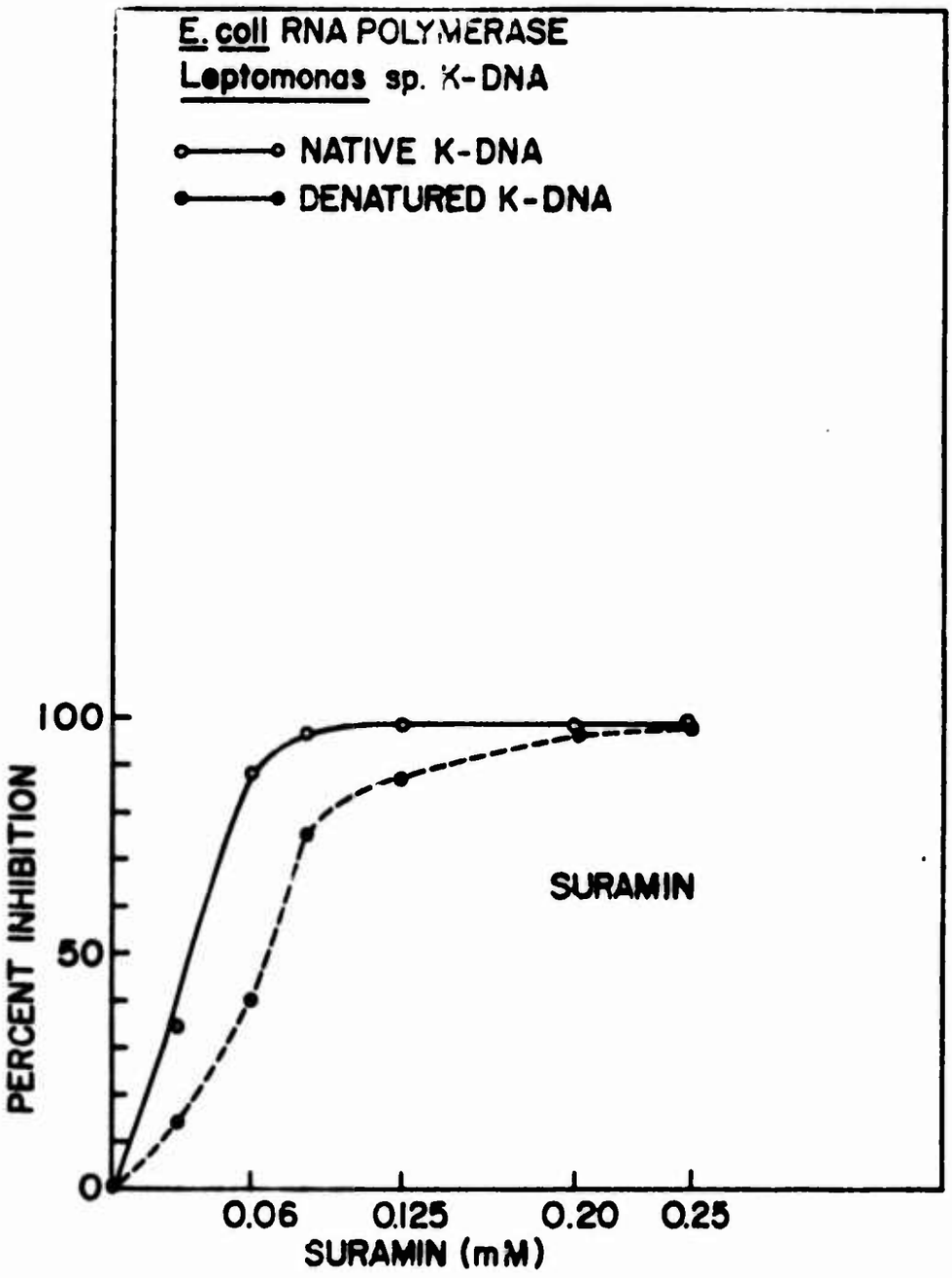

E. coli RNA POLYMERASE
Leptomonas sp. K-DNA
NATIVE K-DNA
DENATURED K-DNA
100
50
0
PERCENT INHIBITION
SURAMIN
0.06
0.125
0.20
0.25
SURAMIN (mM)

FIGURE 8

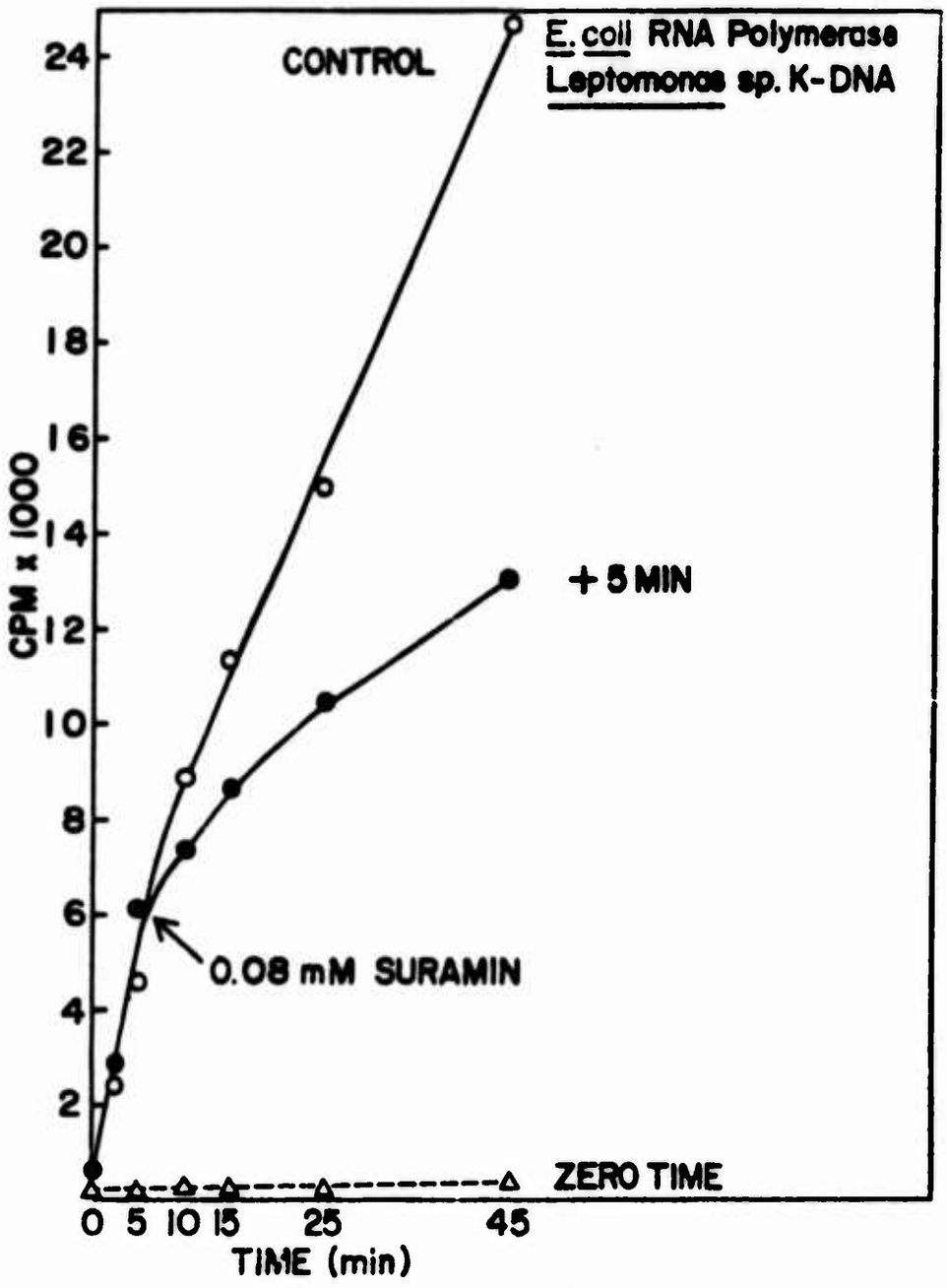

E. coli RNA Polymerase
Leptomonas sp. K-DNA
CONTROL
+5 MIN
0.08 mM SURAMIN
ZERO TIME
CPM x 1000
TIME (min)
0 5 10 15 25 45
2 4 6 8 10 12 14 16 18 20 22 24

FIGURE 9

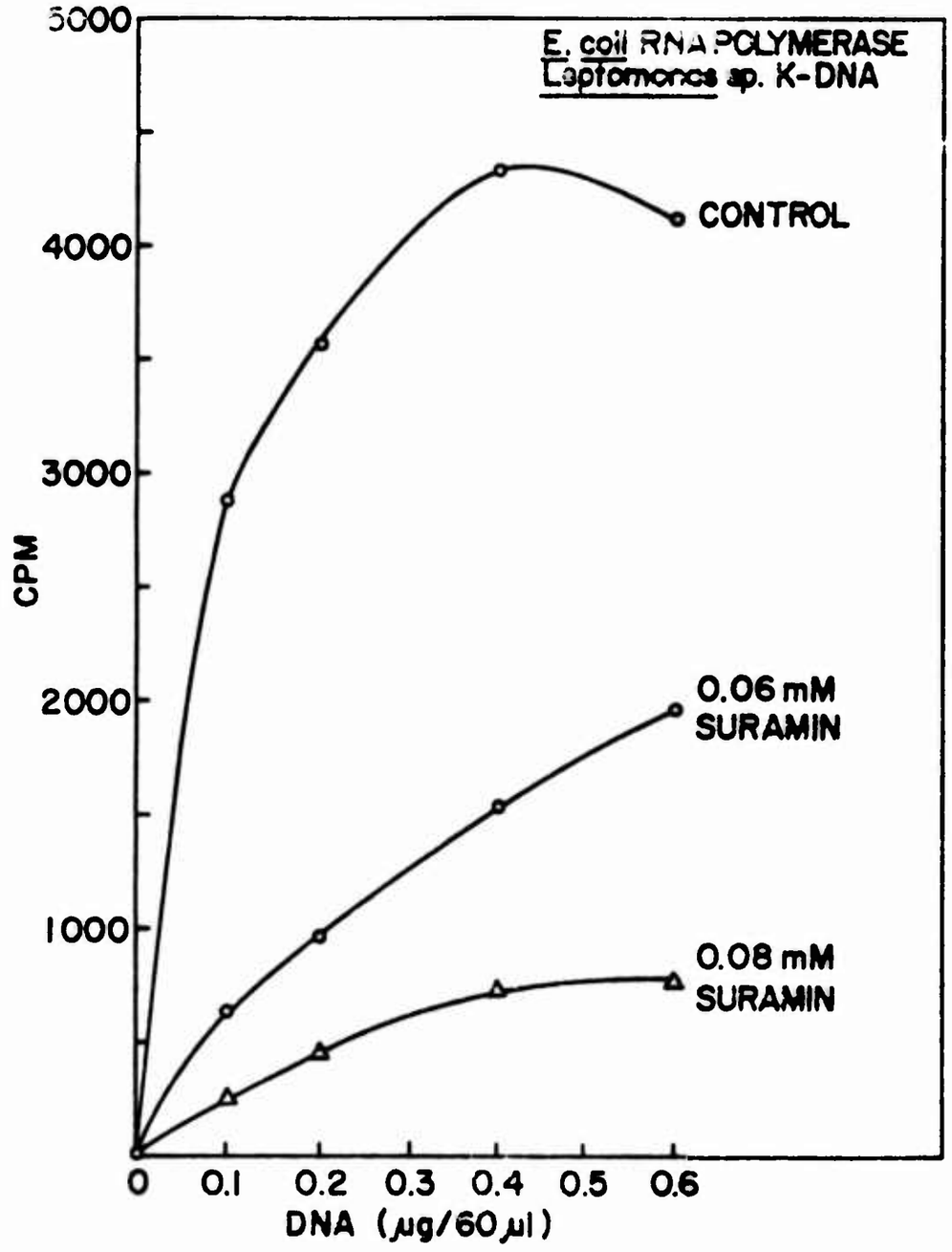

E. coil RNA POLYMERASE
Leptomonas sp. K-DNA
CONTROL
0.06 mM SURAMIN
0.08 mM SURAMIN
CPM
5000
4000
3000
2000
1000
0
0.1
0.2
0.3
0.4
0.5
0.6
DNA (µg/60µl)

FIGURE 10

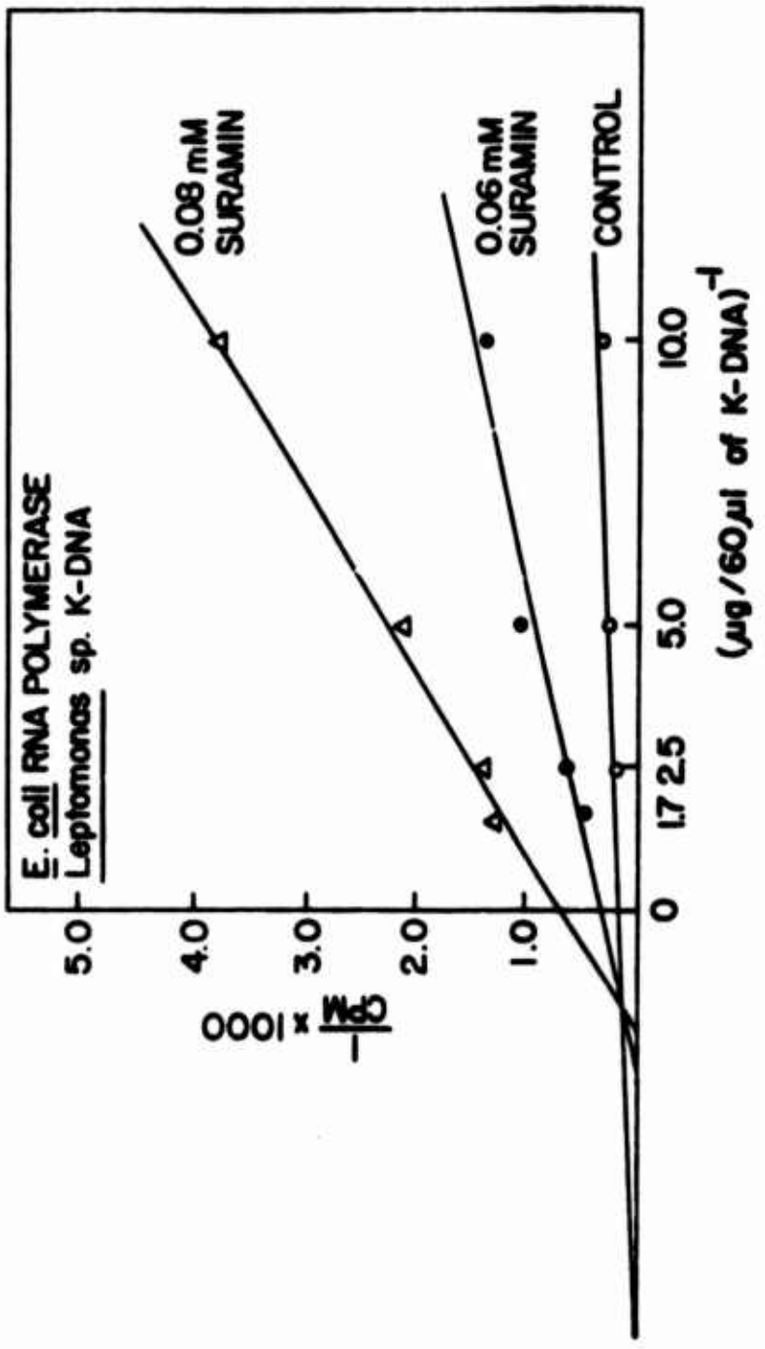

E. coli RNA POLYMERASE
Leptomonas sp. K-DNA
0.08 mM SURAMIN
0.06 mM SURAMIN
CONTROL
5.0
4.0
3.0
2.0
1.0
0
1.7
2.5
5.0
10.0
1/CPM x 1000
(µg/60µl of K-DNA)⁻¹

FIGURE 11

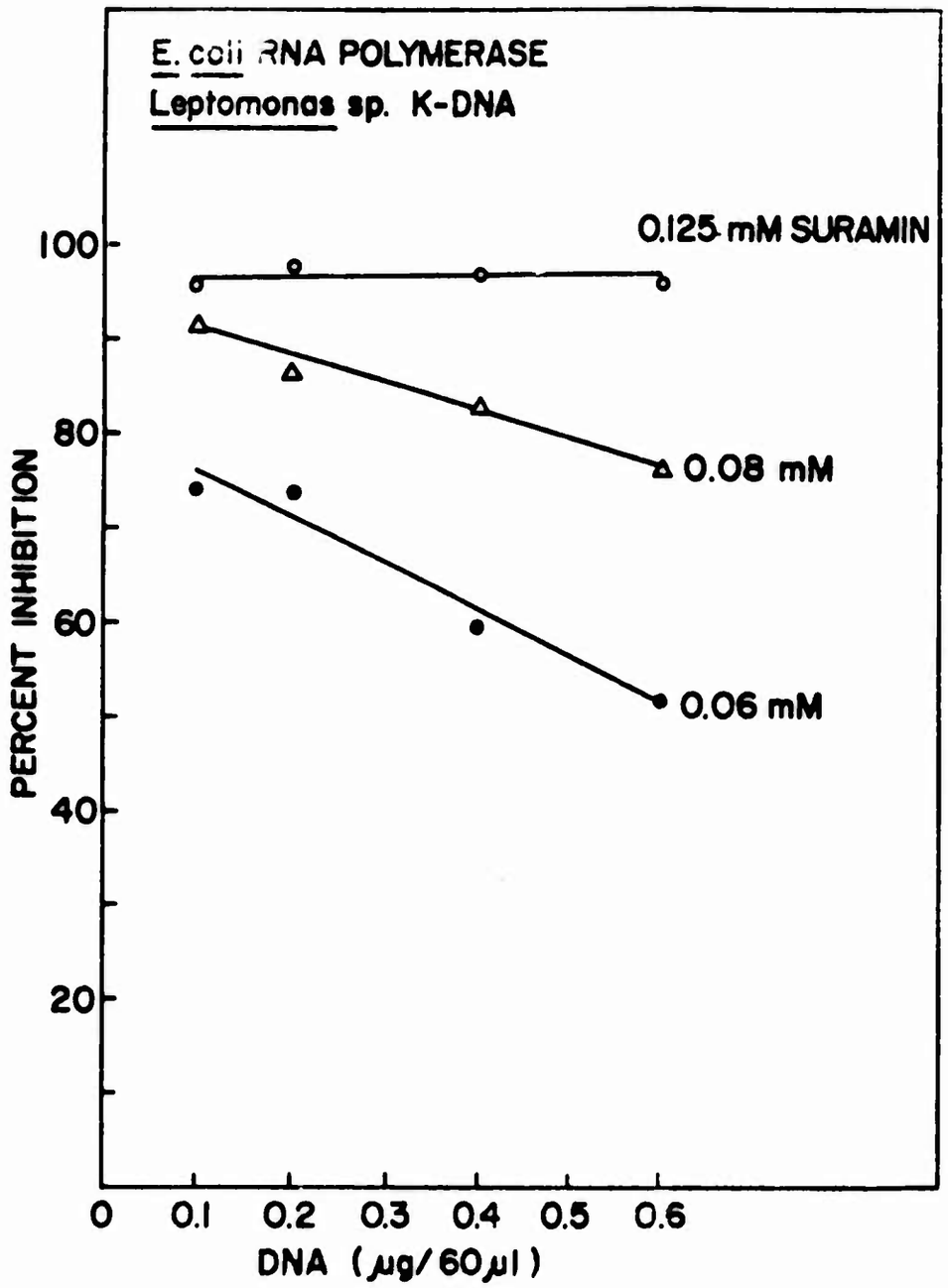

E. coli RNA POLYMERASE
Leptomonas sp. K-DNA
0.125 mM SURAMIN
0.08 mM
0.06 mM
PERCENT INHIBITION
100
80
60
40
20
0
0.1
0.2
0.3
0.4
0.5
0.6
DNA (µg/60µl)

Figure 12

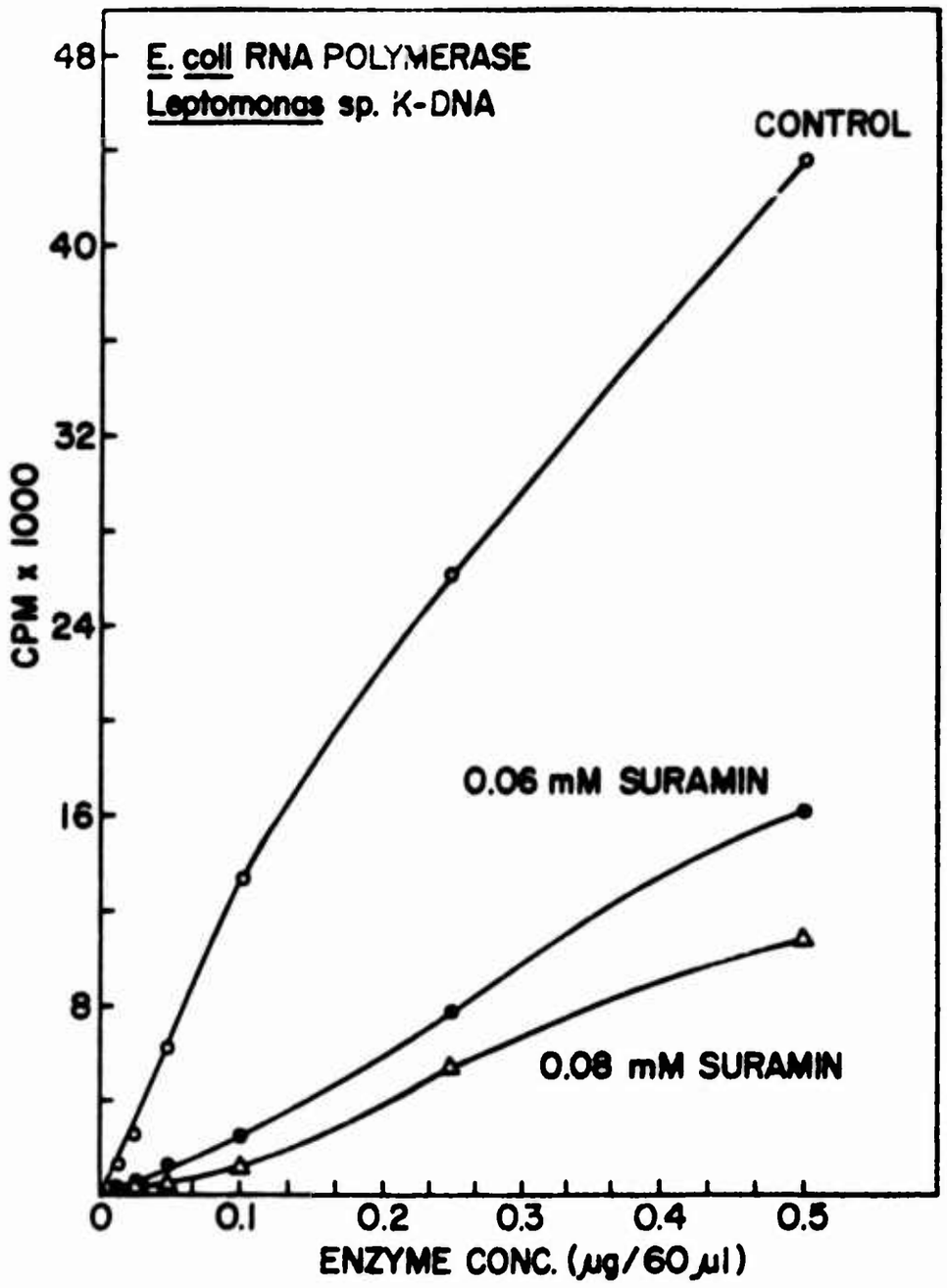

E. coli RNA POLYMERASE
Leptomonas sp. K-DNA
CONTROL
0.06 mM SURAMIN
0.08 mM SURAMIN
CPM x 1000
48
40
32
24
16
8
0
0.1
0.2
0.3
0.4
0.5
ENZYME CONC. (µg/60µl)

FIGURE 13

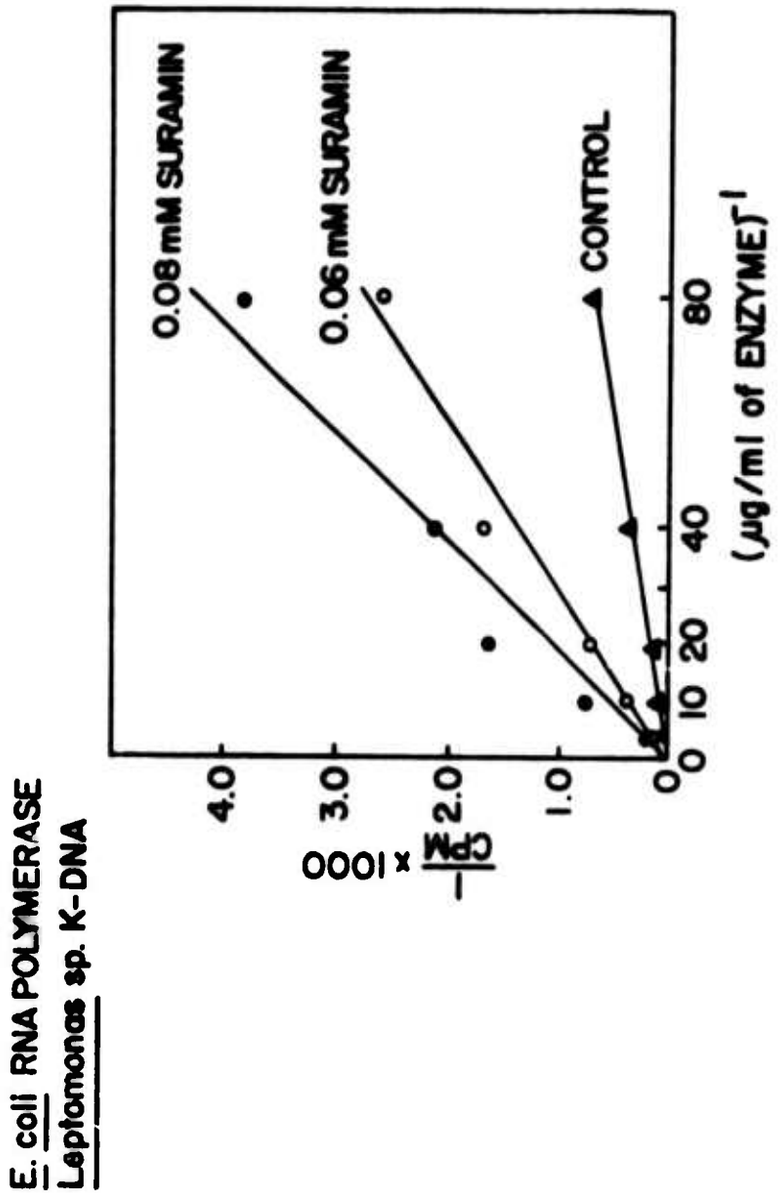

E. coli RNA POLYMERASE
Leptomonas sp. K-DNA
0.08 mM SURAMIN
0.06 mM SURAMIN
CONTROL
4.0
3.0
2.0
1.0
0
0 10 20 40 80
(µg/ml of ENZYME)⁻¹
1/CPM x 1000

Figure 14

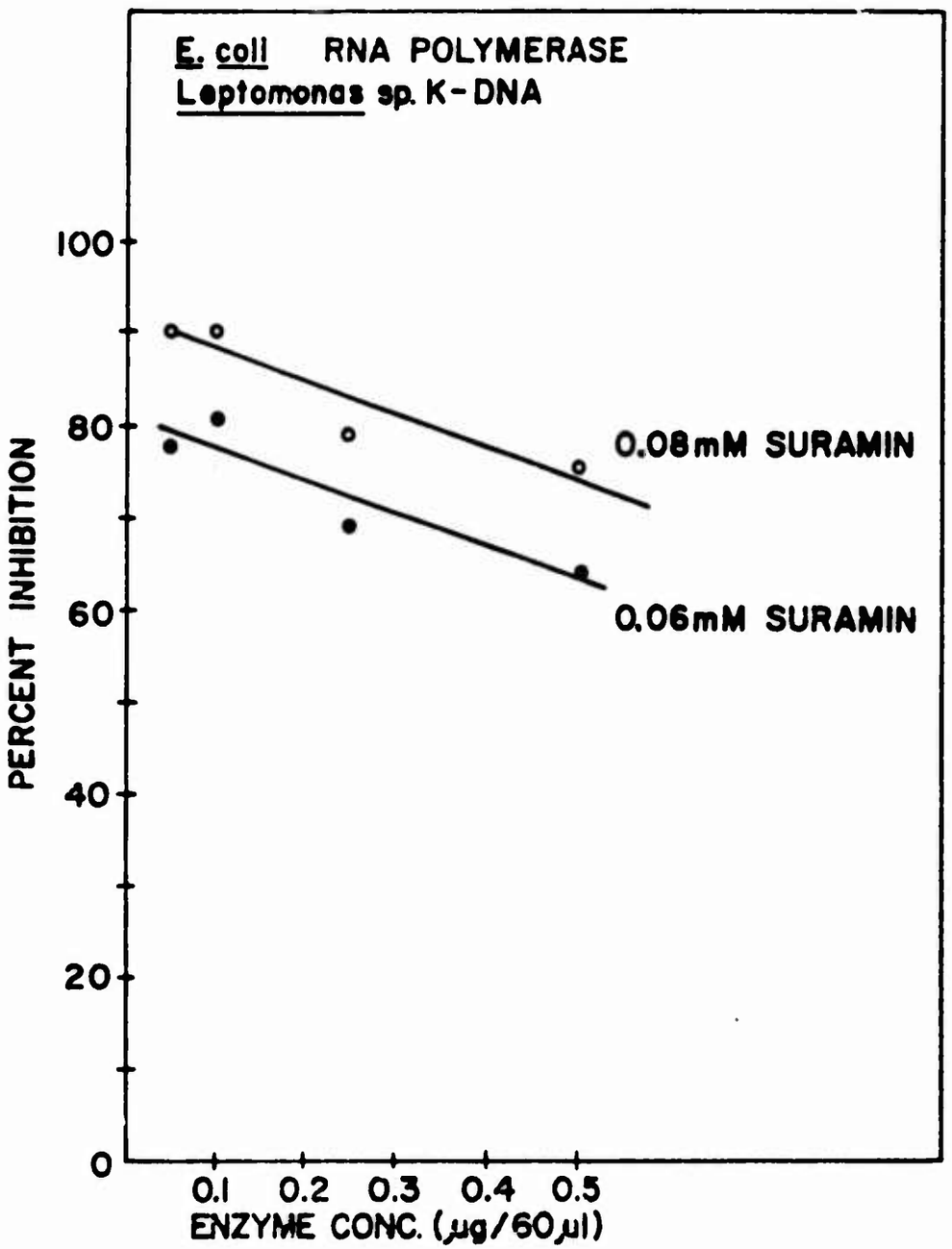
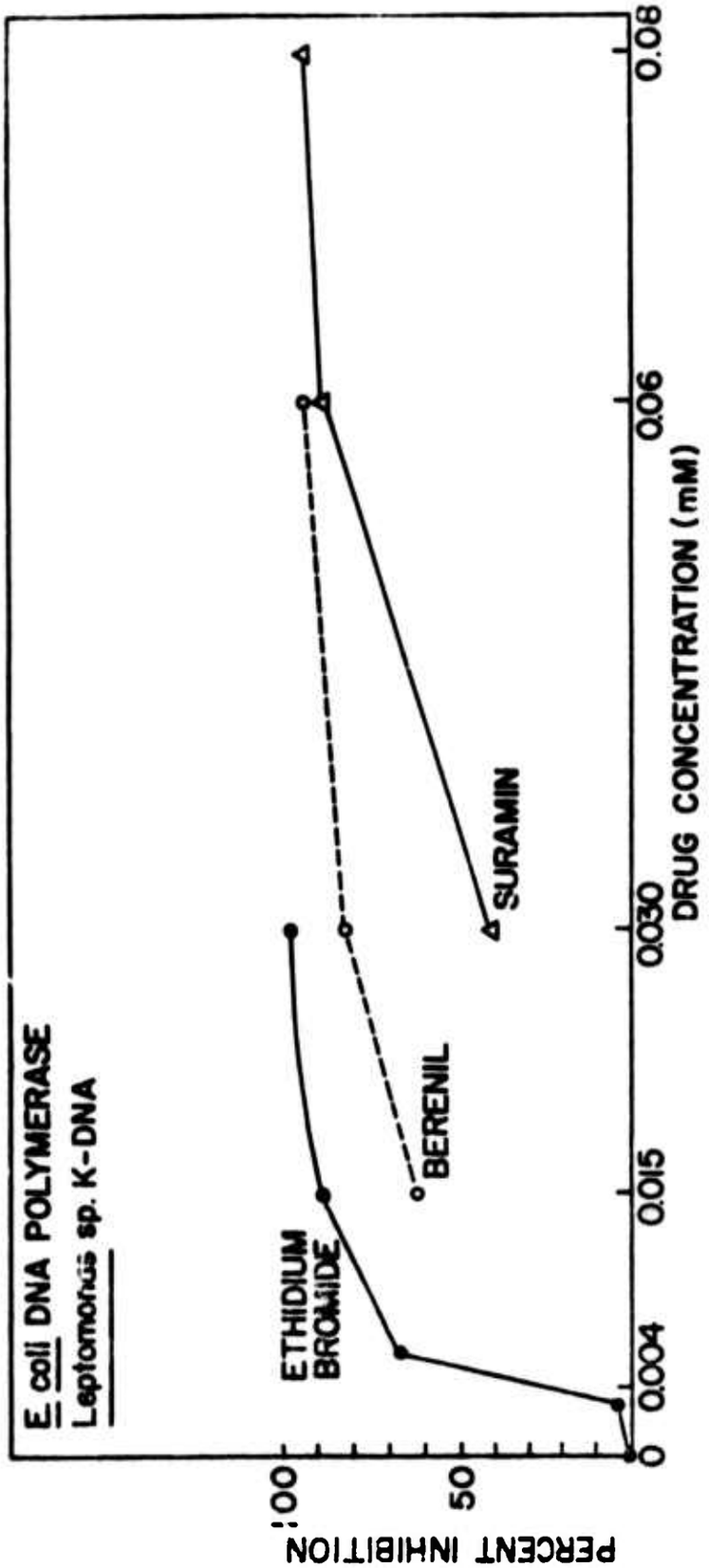

E. coli RNA POLYMERASE
Leptomonas sp. K-DNA
PERCENT INHIBITION
100
80
60
40
20
0
0.08mM SURAMIN
0.06mM SURAMIN
0.1
0.2
0.3
0.4
0.5
ENZYME CONC. (µg/60µl)

Figure 15


E. coli DNA POLYMERASE
Leptomonas sp. K-DNA
ETHIDIUM BROMIDE
BERENIL
SURAMIN
PERCENT INHIBITION
100
50
0
0.004
0.015
0.030
0.06
0.08
DRUG CONCENTRATION (mM)